

Figure 1

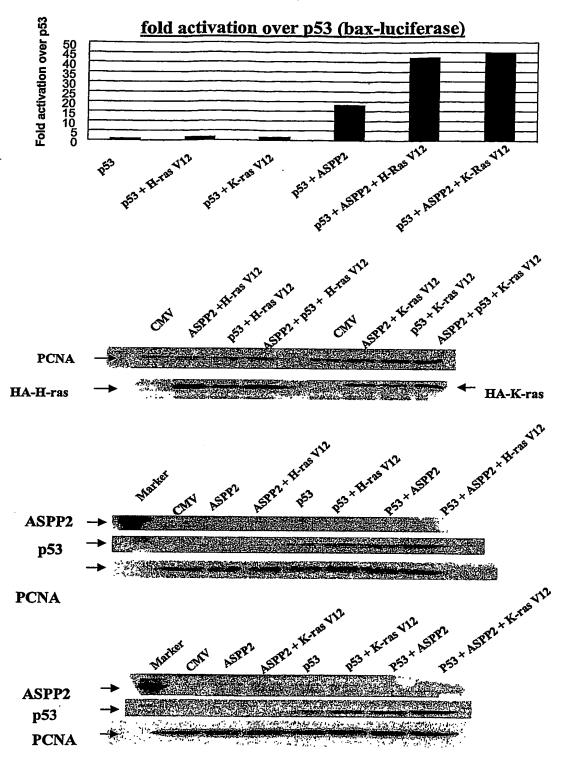
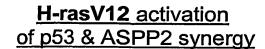
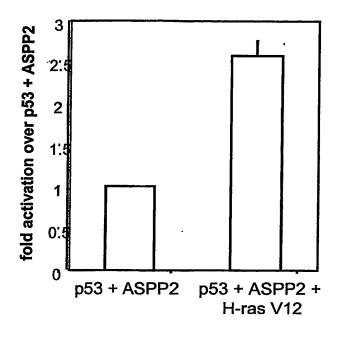


Figure 2

H-ras and K-ras activate ASPP equally



K-rasV12 activation of p53 & ASPP2 synergy



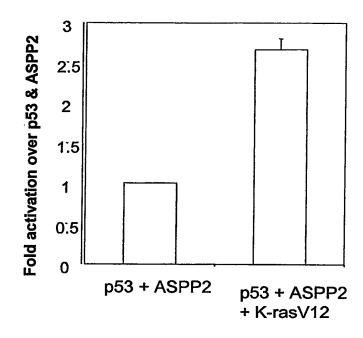


Figure 3

Figure 4A

Promoter specificity

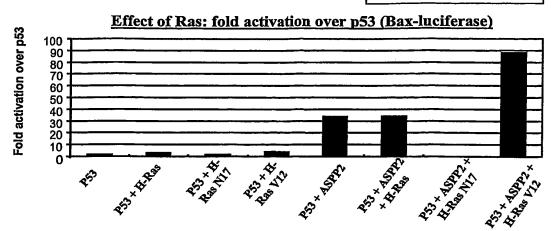


Figure 4B

Effect of Ras Fold activation over p53 (PIG3 reporter)

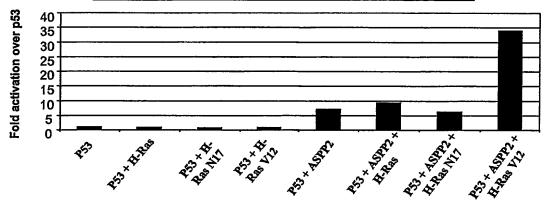


Figure 4C

Effect of Ras: Fold activation over p53 (Mdm2 reporter)

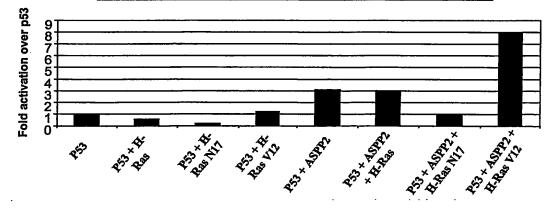
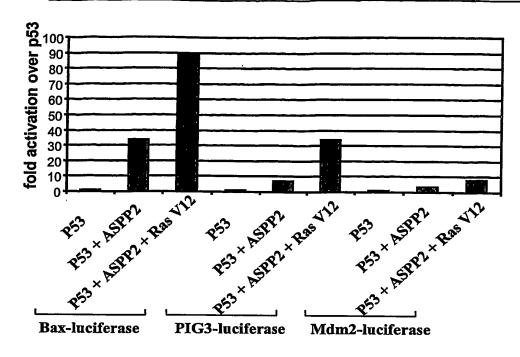


Figure 4D

<u>Effect of rasV12 on transactivation: comparing three reporters</u>



Promoter specificity of rasV12

Mdm2

K-ras

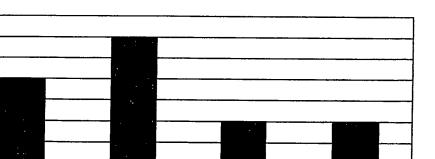
Figure 4E

Fold activation over p53

2.85

2.8 2.75 2.7 2.65 2.6 2.55 2.5

Bax



Bax

H-ras

Mdm2

PCDNA

Figure 5A H-rasV12 activates endogenous ASPP2 to transactivate bax reporter (U2OS cells) 5000 Bax-luciferase/rlu 4500 4000 3500 3000 2500 2000 1500 1000 500 pEF+ pEF+ H-Ras V12 + H-Ras V12 +

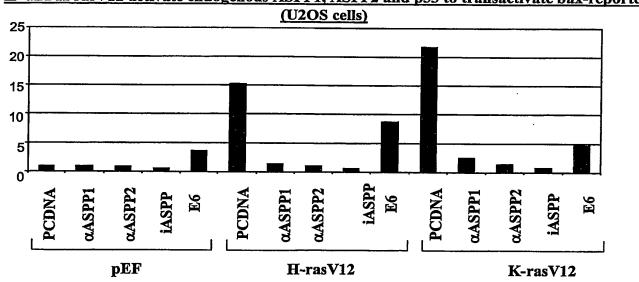
αASPP2

<u>Figure 5B</u>

<u>H- and K-rasV12 activate endogenous ASPP1, ASPP2 and p53 to transactivate bax-reporter</u>

PCDNA

αASPP2



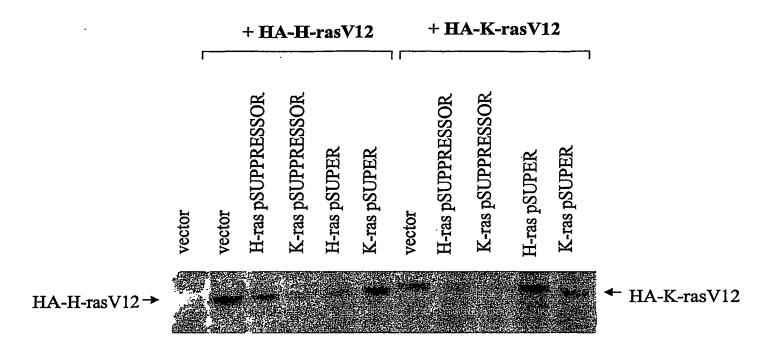
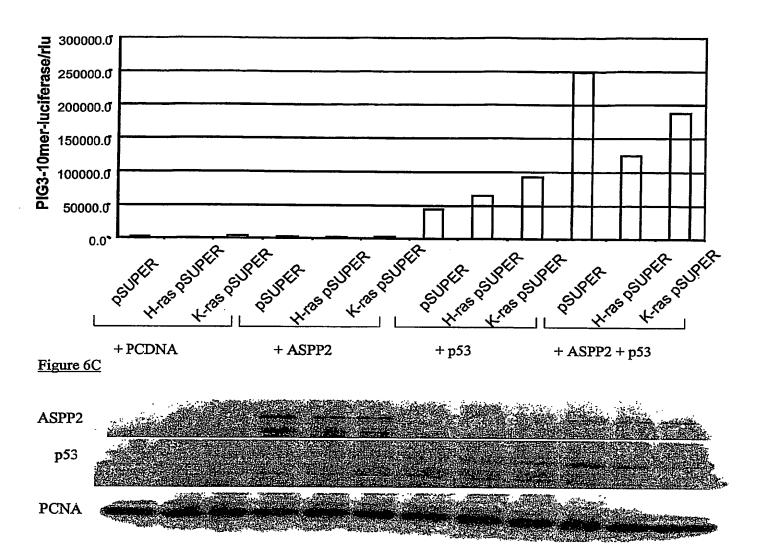


Figure 6

Figure 6B



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Figure 7A

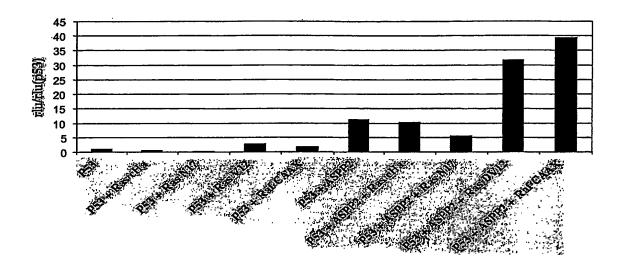


Figure 7B

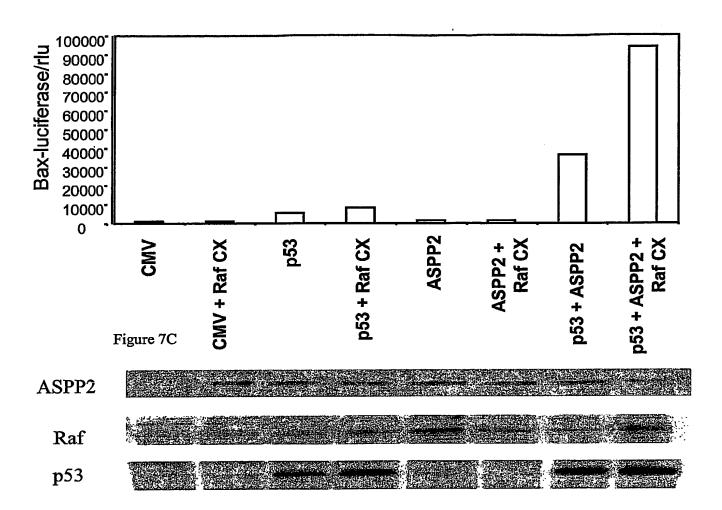
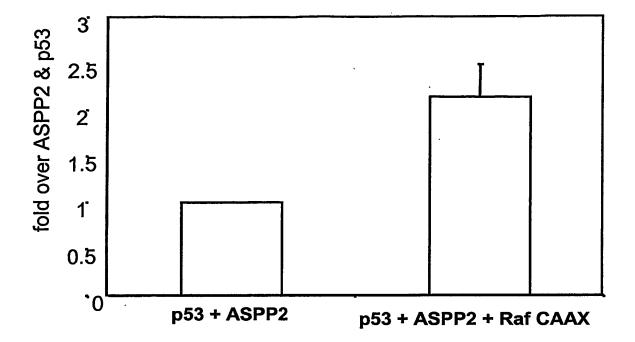
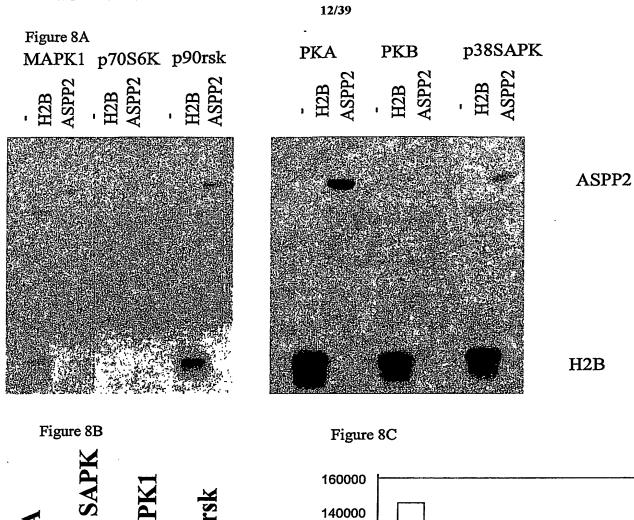
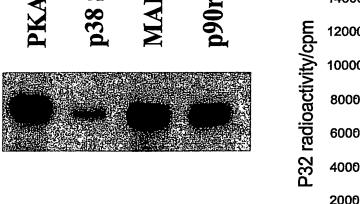


Figure 7d



WO 2005/054862 PCT/GB2004/003899





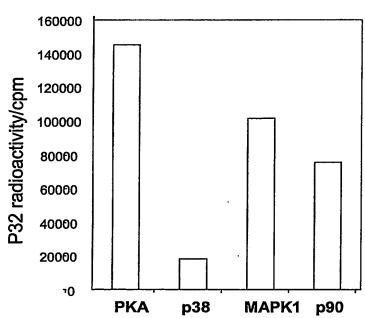
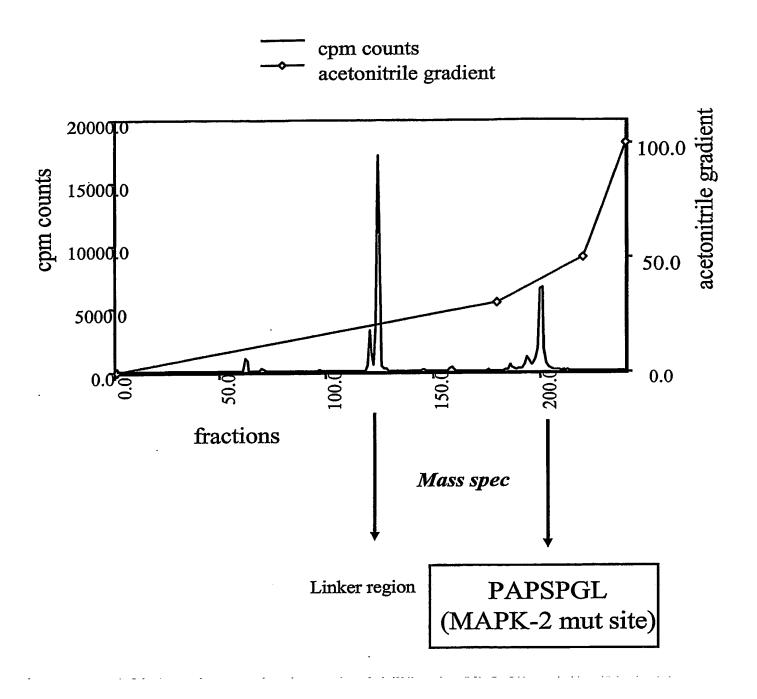


Figure 8D



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Figure 9

C-term of ASPP2:

550 - QPRVLLSPSIPSVGQDQTLSPGSKQESPPAAAVRPFTPQPS
KDTLIPPFRKPQTVAASSIYSMYTQQQAPGKNFQQAVQS
ALTKTHTRCPHFSSVYCKPVIAAAQNQQQHPENIYSNSQ
GKPGSPEPETEPVSSVQENHENERIPRPLSPTKLLPFISNP
YRNQSDADLEALRKKLSNAPRPLKKRSSITEPEGPNCPNI
QKLLYQRTTIAAMETSVPSYPSKSASVTASSESPVEIQNP
YLHVEPEKEVVSLVPESISPEDVCNASTENSDMPAPSPGL
DYEPEGVPDNSPNLQNNPE - 849

S — MAPK sites
SS — PKA site



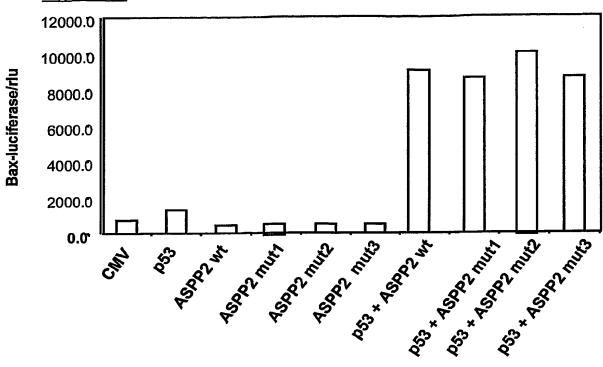


Figure 10B

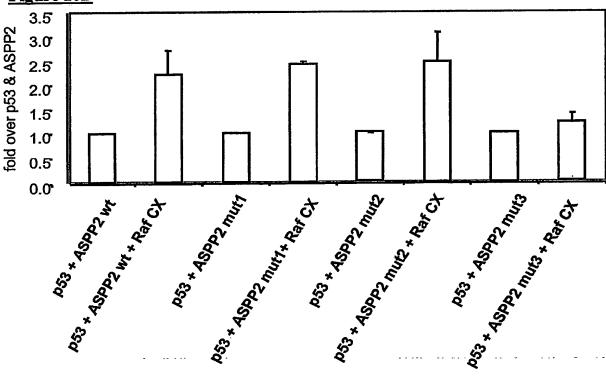
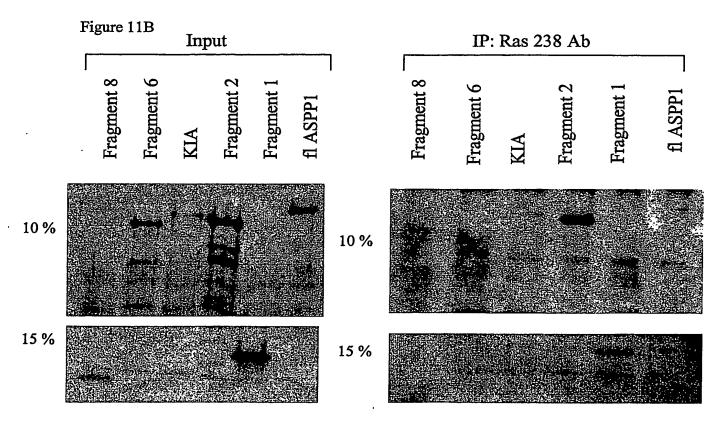


Figure 11A

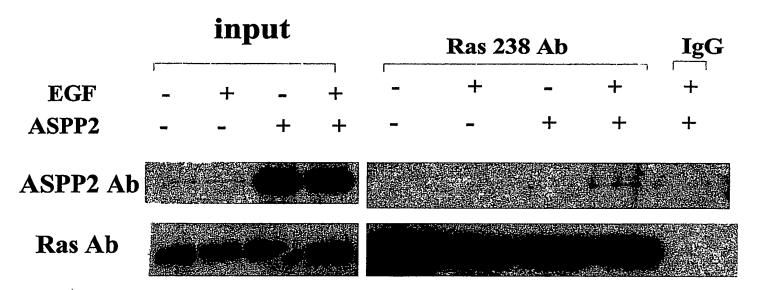
	RAD	Expected binding to ras
	1 143 300 890 1090	
fl ASPP1		+
Fragment 1		+
Fragment 2	2192988888888	+
KIA		?
Fragment 6		-
Fragment 8		-



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Figure 12

Pulldown:



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Figure 13

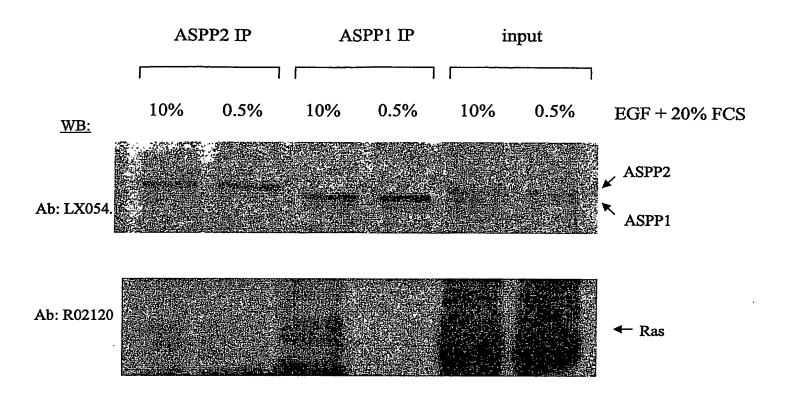
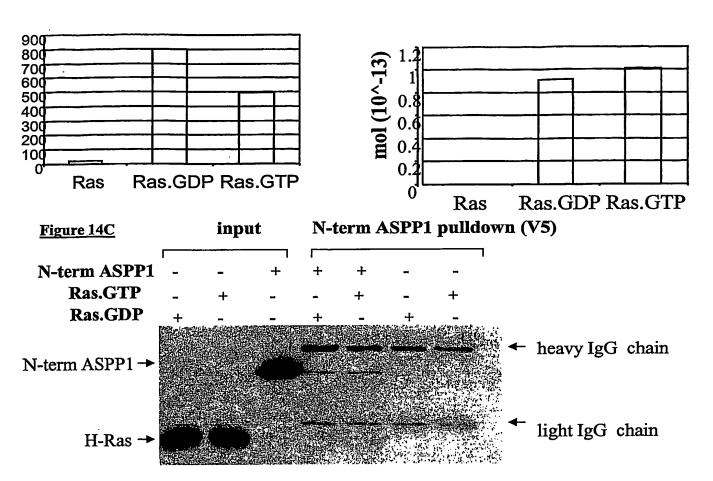


Figure 14A

Figure 14B



ASPP2 + H-RasV12 ASPP2 + H-RasV12 ASPP2 H-RasV12 Merged

Figure 15

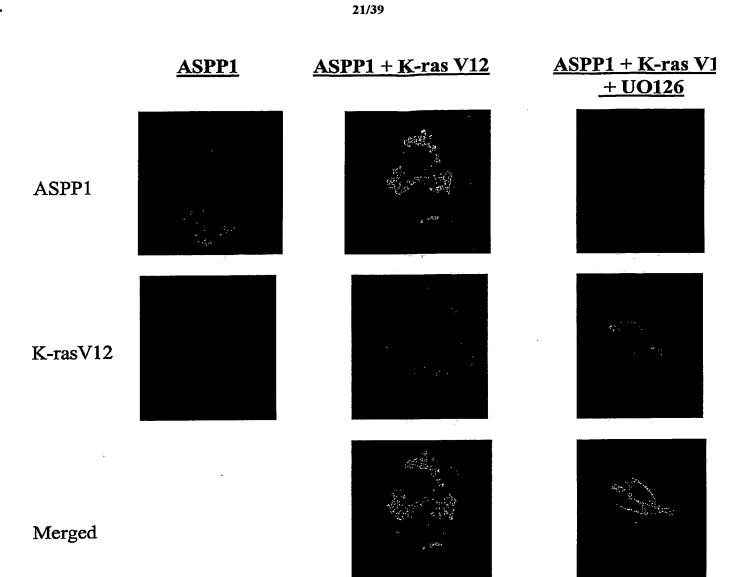


Figure 16

Figure 17a

CGGCCGGAGCGGTGGGCACAGCTCGGCGCGGAGCGTCCTGTCAGGCGGCGGCGGCGAGGGCGTCGCGGACTCTCCCCGCGAT GATGCCGATGATATTAACTGTTTTCTTGAGCAACAATGAACAGATTTTAACAGAAGTTCCTATAACACCGGAAACAACCT GTCGAGATGTTGTAGAATTTTGCAAGGAACCTGGAGAAGGCAGCTGCCATTTAGCTGAAGTGTGGAGGGGAAATGAACGT CCCATACCCTTTGATCATATGATGTACGAACATCTTCAGATATGGGGTCCACGGAGGGAAGAAGTGAAATTTTTCCTTCG acacgaggactccccaactgagaacagtgaacaaggtggccgtcagacccaagagcaacgaactcagagaaatgtaataa ATGTACCTGGAGATAAACGTACTGAATATGGGGTTGGGAATCCACGTGTTGAACTTACCCTCTCAGAGCTCCAAGATATG ACAGGAGCGCCGTCAGCAGCAGTCTATTTCTGAAAATGAAAAGCTTCAGAAATTGAAAGCAGCTCAGAGAGA GAAAGGTTCAGTGCCATGTTCCAGGAAAAGAAGCAGGAAGTACAGACTGCAATTTTAAGGGTTGATCAGCTTAGTCAGCA ATTGGAAGATTTAAAGAAAGGAAAACTGAATGGGTTCCAGTCTTACAATGGCAAATTGACGGGACCAGCGGCGGTGGAGT TAAAAAGACTGTACCAAGAACTACAGATTCGTAACCAACTTAACCAGGAACAAAATTCAAAAACTTCAGCAGCAGAAGGAA CTCTTAAATAAGCGCAACATGGAGGTGGCCATGATGGACAAGCGAATCAGTGAACTGCGTGAACGTCTCTATGGGAAAAA AATTCAGCTGAACCGTGTGAATGGCACGTCATCACCACAGTCCCCTCTGAGCACATCGGGCAGGGTCGCTGCTGTGGGGC CTTATATCCAGGTTCCCAGTGCCGGAAGCTTTCCTGTGCTGGGGGACCCTATAAAGCCCCAGTCTCTCAGTATTGCCTCA AATGCTGCTCATGGAAGATCCGAAATCCGCTAATGATGGAAACTGGCCAACATTAAAACAGAAATTCTAGCTCTTCCGTGAA CTGTGCCCTTCTCAGCACTGGGACCCACGGAGAAGCCGGGCATCGAGATTGGTAAAGTGCCACCTCCCATCCCGGGTGTA GGCAAGCAGCTGCCCAAGCTATGGGACATACCCAAGTCCTACACCTCTGGGTCCTGGGTCGACAAGCTCCCTGGAAAG GAGGAAGGAAGGCAGCTTGCCCAGGCCCAGTGCAGGCCTGCCAAGTCGACAGAGGCCCACCCTGCTGCCCGCCACAGGCA GCACCCCCAGCCAGGCTCCTCACAACAGATTCAGCAGAGGATTTCCGTACCGCCAAGTCCCACGTACCCGCCAGCGGGA CCACCTGCATTTCCAGCTGGGGACAGCATGAACTCCCACTGACAGTGGCCATTAGGCCTTTCCTGGCTGATAAAGG GTCAAGGCCACAGTCTCCCAGGAAAGGACCCCAGACAGTGAATTCAAGTTCCATATACTCCATGTACCTCCAGCAAGCCA CACCACCTAAGAATTACCAGCCGGCAGCACACAGCGCCTTAAATAAGTCAGTTAAAGCAGTGTATGGTAAGCCCGTTTTA CCTTCGGGTTCAACCTCTCCATCGCCGCTGCCGTTTCTTCACGGGTCACTGTCCACGGGCACACCACAGCCTCAGCCACC TTCAGAAAGTACTGAGAAAGAGCCTGAGCAGGATGGCCCCGCCCCCCCGCAGATGGCACCCGTGGAGAGCCTGCCAC GGCCACTCAGCCCCACCAAGCTCACGCCCATCGTGCATTCGCCACTGCGCTACCAGAGTGATGCAGACCTGGAGGCCTTC CATCCAGAAGCTGCTGTACCAGCGCTTCAACACCCTGGCCGGTGGCATGGAGGGCACCCCTTTCTACCAGCCCAGCCCCT CCCAGGACTTCATGGGCACCTTGGCCGATGTGGACAATGGAAACACCAATGCCAATGGAAAACCTGGAAGAGCTCCCCCCT GCCCAGCCCACAGCCCCACTCCCCGCTGAGCCTGCCCCGTCATCAGATGCCAATGATAATGAGTTACCTTCCCCCGAACC AGAGGAGCTCATCTGTCCCCAAACCACCCACCAAACTGCCGAGCCGGCAGAGGACAATAACAACAACGTGGCCACGGTCC CCTGCCAGCCACCTCCTGCCACCTCCACGAACAAGCGGACCAACTTGAAGAAGCCCAACTCGGAGCGGACCGGGCACGG GCTGAGAGTCCGGTTTAACCCCCTGGCACTGCTCCTAGACGCGTCTCTGGAAGGAGAGTTCGATCTGGTGCAGAGGATCA TCTATGAGGTGGAAGATCCCAGCAAGCCCAACGATGAAGGGATCACCCCACTGCACAACGCCGTCTGCGCCGGCCACCAT CACATCGTGAAGTTCCTGCTGGATTTTGGTGTCAACGTGAATGCTGCTGATAGTGATGGACGCCGCCGCTGCACTGCGC TGCCTCTTGTAACAGCGTTCACCTCTGCAAACAGCTGGTGGAGAGTGGTGCCGCCATTTTTGCCTCAACCATAAGCGACA TTGAAACTGCTGCAGACAAGTGTGAGGAGATGGAGGAGGCTACATCCAGTGCTCCCAGTTTCTATATGGGGTGCAGGAA AAGCTGGGTGTGAACAAAGGTGTGGCGTATGCTCTGTGGGACTACGAGGCCCAGAACAGTGACGAGCTGTCCTTCCA CGAAGGGGACGCCTCACCATCCTGAGGCGCAAGGACGAAGGCGAGACTGAGTGGTGGTGGTCGCCTTGGAGACCGGG AGGGCTATGTGCCCAAAAACCTGCTGGGGCTGTATCCACGGATCAAACCCCGACAGCGAACACTCGCCTGAACTTCCTTT AAAATGGTCTTAATGGTGCTCACTTTAGCAGACAGCGTCCACAATGTGAATCCTACAGTTTCCAGGTGAGGCCCTTTCTC TACTGACTTGGCCCCGAGGCCATCACCCCCTCCAGCAGTGAACACTGTCCGCCGCTGTGAGGCCTGCTCCCCTGCGACCG $\tt CCCTGCCCCGTCACCGAATCGGACACTCATCCTTTCTCACACTTCCCACACATGATCCTTCTTCCCTTCATCACCAAA$ GGGGTTCCGCTTCCTGTTCCAGTTCACCTAAAGGCTGATTGTGCAGGCCCAGCACTGTGGCTGGACTGCCGCGCCCACGGG CACCAGGACCCCTAAGACCAAGTGACAACTGGGAGAGCCTCAGCATATACTCTTCTCCTCCGATCTCACAGCCTGTCATG CTGCTCAGTGTGGTTCTCACCCCTGCAAGCTCAAATTCAGTTCCCTGAATGGAGTCAGGTGCTGGAGGCCGTGGCAGCGG AGGGTGGTTGGGGCTGGGGTGGACTGGTGTGAGGGCAGACCAGGGCCAGGTAGACGGGGCTGTTTGGTGCCTG AAGGATGGCAGACGCCTGGTGTCAGGAGGGGCCCCACCAAGGAGCAGCTGGGGCAGAGGAGCAGCTGGGGTCAGGGGCC GCTTCACTGAGGTGTGAATTGTACGTACAGGCTTTTTATATACCAAAAGTATTTTTTGACTAGACCATTCAAAGCTACCC GAACTATGTTGGAAATTTTTTTTTTTTTTTTTAAAATACAGGCCCTTAGGCTCTATTTTTTCATGTATGAGTCGTGTGTAA GTGAAGAAAAGTGAACGCCCTTGTAGAGCAGCCCGACCACAGGAGCATGGCCGCTGCCAGACGCTGCTGACGCTG TGTAAATGTGCACAATAAACCCGTCTCACCCCGG

. . .

Figure 17b

CCGCGCGGAGGCCCTTCGGACCCGCGCCGCCGCCGCCGCCGCCGCCTCGCAACAGGTCCGGGCGCCTCGCTCT CCGCTCCCCTCCCCGCATCCGCGACCCTCCGGGGCACCTCAGCTCGGCCGGGGCCGCAGTCTGGCCACCCGCTTCCATG CGGTTCGGGTCCAAGATGATGCCGATGTTTCTTACCGTGTATCTCAGTAACAATGAGCAGCACCTTCACAGAAGTTCCAGT TACTCCAGAAACAATATGCAGAGACGTGGTGGATCTGTGCAAAGAACCCGGCGAGAGTGATTGCCATTTGGCTGAAGTGT GGTGTGGCTCTGAACGTCCAGTTGCGGATAATGAGCGAATGTTTGATGTTCTTCAACGATTTGGAAGTCAGAGGAACGAA GTTCGCTTCTTCCTTCATGAACGCCCCCTGGCAGGACATTGTGAGTGGACCAAGATCTCAGGATCCAAGTTTAAA CTGAACTTCAGGAAATGGCATCTCGCCAGCAGCAACAGATTGAAGCCCAGCAACAATTGCTGGCAACTAAGGAACAGCGC TTAAAGTTTTTGAAACAACAAGATCAGCGACAACAAGCAACAAGTTGCTGAGCAGGAGAAACTTAAAAGGCTAAAAAGAAAT AGCTGAGAATCAGGAAGCTAAGCTAAAAAAAGTGAGAGCACTTAAAGGCCACGTGGAACAGAAGAGACTAAGCAATGGGA AACTTGTGGAGGAAATTGAACAGATGAATAATTTGTTCCAGCAAAAACAGAGGGAGCTCGTCCTGGCTGTCTCAAAAAGTA GAAGAACTGACCAGGCAGCTAGAGAATGCTCAAGAACGGCAGGATCGACCACCATGACAATCAGTCTGCAGTGGCTGA GCTTGATCGCCTCTATAAGGAGCTGCAGCTAAGAAACAAATTGAATCAAGAGCAGAATGCCAAGCTACAACAACAGAGGG AGTGTTTGAATAAGCGTAATTCAGAAGTGGCAGTCATGGATAAGCGTGTTAATGAGCTGAGGGACCGGCTGTGGAAGAAG AAGGCAGCTCTACAGCAAAAAGAAAATCTACCAGTTTCATCTGATGGAAATCTTCCCCAGCAAGCCGCGTCAGCCCCAAG CCGTGTGGCTGCAGTAGGTCCCTATATCCAGTCGTCTACTATGCCTCGGATGCCCTCAAGGCCTGAATTGCTGGTGAAGC CAGCCCTGCCGGATGGTTCCTTGGTCATTCAGGCTTCAGAGGGGCCGATGAAAATACAGACACTGCCCAACATGAGATCT GGGGCTGCTTCACAAACTAAAGGCTCTAAAATCCATCCAGTTGGCCCTGATTGGAGTCCTTCAAATGCAGATCTTTTCCC AAGCCAAGGCTCTGCTTCTGTACCTCAAAGCACTGGGAATGCTCTGGATCAAGTTGATGATGAGGAGGTTCCGCTGAGGG AGGAAGAACCAGAGCAGTGAAGATATCTTGCGGGATGCTCAGGTTGCAAATAAAAATGTGGCTAAAGTACCACCTCCTGT TCCTACAAAACCAAAACAGATTAATTTGCCTTATTTTGGACAAACTAATCAGCCACCTTCAGACATTAAGCCAGACGGAA GTTCTCAGCAGTTGTCAACAGTTGTTCCGTCCATGGGAACTAAACCAAAACCAGCAGGGCAGCAGCCGAGAGTGCTGCTA TCTCCCAGCATACCTTCGGTTGGCCAAGACCAGACCCTTTCTCCAGGTTCTAAGCAAGAAAGTCCACCTGCTGCTGCCGT CCGGCCCTTTACTCCCCAGCCTTCCAAAGACACCTTACTTCCACCCTTCAGAAAACCCCAGACCGTGGCAGCAAGTTCAA TATATTCCATGTATACGCAACAGCAGGCGCCAGGAAAAAACTTCCAGCAGGCTGTGCAGAGCGCGTTGACCAAGACTCAT ACCAGAGGGCCACACTTTCAAGTGTATATGGTAAGCCTGTAATTGCTGCTGCCCAGAATCAACAGCAGCACCCAGAGAA CATTTATTCCAATAGCCAGGGCAAGCCTGGCAGTCCAGAACCTGAAACAGAGCCTGTTTCTTCAGTTCAGGAGAACCATG AAAACGAAAGAATTCCTCGGCCACTCAGCCCAACTAAATTACTGCCTTTCTTATCTAATCCTTACCGAAACCAGAGTGAT GCTGACCTAGAAGCCTTACGAAAGAAACTGTCTAACGCACCAAGGCCTCTAAAGAAACGTAGTTCTATTACAGAGCCAGA GGGTCCTAATGGGCCAAATATTCAGAAGCTTTTATATCAGAGGACCACCATAGCGGCCATGGAGACCACCAT CATACCCATCCAAGTCAGCTTCTGTGACTGCCAGCTCAGAAAGCCCAGTAGAAATCCAGAATCCATATTTACATGTGGAG CCCGAAAAGGAGGTGGTCTCTCTGGTTCCTGAATCATTGTCCCCAGAGGATGTGGGGAATGCCAGTACAGAGAACAGTGA CATGCCAGCTCCTTCTCCAGGCCTTGATTATGAGCCTGAGGGGAGTCCCAGACAACAGCCCAAATCTCCAGAATAACCCAG TGGTAAAAGGACAAACTTGCGTAAAACTGGCTCAGAGCGTATCGCTCATGGAATGAGGGTGAAATTCAACCCCCTTGCTT tgtabatgtaaatgctgctgatagtgatggatggactccattacattgtgctgcctcatgtaacaacgtccaagtgtgta AGTTTTTGGTGGAGTCAGGAGCCGCTGTGTTTTGCCATGACCTACAGTGACATGCAGACTGCTGCAGATAAGTGCGAGGAA ATGGAGGAGGCTACACTCAGTGCTCCCAATTTCTTTATGGAGTTCAGGAGAAGATGGGCATAATGAATAAAGGAGTCAT TTATGCGCTTTGGGATTATGAACCTCAGAATGATGAGGGGGCCCATGAAAGAAGGAGACTGCATGACAATCATCACA GGGAAGACGAAGATGAAATCGAATGGTGGTGGGCGCCCTTAATGATAAGGAGGGATATGTTCCACGTAACTTGCTGGGA CTGTACCCAAGAATTAAACCAAGACAAAGGAGCTTGGCCTGAAACTTCCACACAGAATTTTAGTCAATGAAGAATTAATC TCTGTTAAGAAGAAGTAATACGATTATTTTTGGCAAAAATTTCACAAGACTTATTTTAATGACAATGTAGCTTGAAAGCG ATGAAGAATGTCTCTAGAAGAATGAAGGATTGAAGAATTCACCATTAGAGGACATTTAGCGTGATGAAATAAAGCATC TACGTCAGCAGGCCATACTGTGTTGGGGCAAAGGTGTCCCGTGTAGCACTCAGATAAGTATACAGCGACAATCCTGTTTT CTACAAGAATCCTGTCTAGTAAATAGGATCATTTATTGGGCAGTTGGGAAATCAGCTCTCTGTCCTGTTGAGTGTTTTCA GCAGCTGCTCCTAAACCAGTCCTCCTGCCAGAAAGGACCAGTGCCGTCACATCGCTGTCTCTGATTGTCCCCGGCACCAG TGAACAATAACTTTATTATATGAGTTTTTGTAGCATCTTAAGAATTATACATATGTTTGAAATATTGAAACTAAGCTACA GAAACTTGCTACAGACTTACCCGTAATATTTGTCAAGATCATAGCTGACTTTAAAAACAGTTGTAATAAACTTTTTTGATG

Figure 17c

MMPMILTVFLSNNEQILTEVPITPETTCRDVVEFCKEPGEGSCHLAEVWRGNERPIPFDHMMYEHLQIWGPRREEVKFFL RHEDSPTENSEQGGRQTQEQRTQRUVINVPGDKRTEYGVGNPRVELTLSELQDMAARQQQQIENQQQMLVAKEQRLHFLK QQERRQQQSISENEKLQKUKERVEAQENKLKKIRAMRGQVDYSKIMMGNLSABIERFSAMFQEKKQEVQTAILRVDQLSQ QLEDLKKGKLNGFQSYNGKLTGPAAVELKRLYQELQIRNQLNQEQNSKLQQQKELLNKRNMEVAMMDKRISELRERLYGK KIQLNRVNGTSSPQSPLSTSGRVAAVGPYIQVPSAGSFPVLGDPIKPQSLSIASNAAHGRSKSANDGNWPTLKQNSSSSV KPVQVAGADWKDPSVEGSVKQGTVSSQPVPFSALGPTEKPGIBIGKVPPPIPGYGKQLPPSYGTYBSPTPLGPGSTSSLE RRKEGSIPRPSAGLPSRQRPTLLPATGSTPQPCSSQQIQQRISVPPSPTYPPAGPPAFPAGDSKPELPLTVAIRPFLADK GSRPQSPRKGPQTVNSSSIYSMYLQQATPPKNYQPAAHSALNKSVKAVYGKPVLPSGSTSPSPLPFLHGSLSTGTPQPQP PSESTEKEPBQDGPAAPADGSTVESLPRPLSPTKLTPIVHSPLRYQSDADLEALRRKLANAPRPLKKRSSITEPBGPGGP NIQKLLYQRFNTLAGGMEGTPFYQPSPSQDFMGTLADVDNGNTMANGNLEELPPAQPTAPLPAEPAPSSDANDNELPSPE PEELICPQTTHQTABPAEDNNNNVATVPTTEQIPSPVAEAPSPGEEQVPPAPLPPASHPPATSTNKRTNLKKPNSERTGH GLRVRFNPLALLDASLEGEFDLVQRIIYEVEDPSKPNDEGITPLHNAVCAGHHHIVKFLLDFGVNVNAADSDGWTPLHC AASCNSVHLCKQLVESGAAIFASTISDIETAADKCEEMEEGYIQCSQFLYGVQEKLGVMNKGVAYALWDYEAQNSDELSF HEGDALTTLRKDESETEWWWARLGDREGYVPKNLLGLYPRIKPRQRTLA

Figure 17d

MMPMFLTVYLSNNEQHFTEVPVTPETICRDVVDLCKEPGESDCHLAEVWCGSERPVADNERMFDVLQRFGSQRNEVRFFL
RHERPPGRDIVSGPRSQDPSLKRNGVKVPGEYRRKENGVNSPRMDLTLAELQEMASRQQQIEAQQQLLATKEQRLKFLK
QDQRQQQQVAEQEKLKRLKEIAENQEAKLKKVRALKGHVEQKRLSNGKLVEEIEQMNNLFQQKQRELVLAVSKVEELTR
QLEMLKNGRIDSHHDNQSAVAELDRLYKELQLRNKLNQEQNAKLQQRECLNKRNSEVAVMDKRVNELRDRLWKKKAALQ
QKENLPVSSDGNLPQQAASAPSRVAAVGPYIQSSTMPRMPSRPELLVKPALPDGSLVIQASEGPMKIQTLPNMRSGAASQ
TKGSKIHPVGPDWSPSNADLFPSQGSASVPQSTGNALDQVDDGEVPLREKEKKVRPFSMFDAVDQSNAPPSFGTLRKNQS
SEDILRDAQVANKNVAKVPPPVPTKPKQINLPYFGQTNQPPSDIKPDGSSQQLSTVVPSMGTKPKPAGQQPRVLLSPSIP
SVGQDQTLSFGSKQESPPAAAVRPFTPQPSKDTLLPPFRKPQTVAASSIYSMYTQQQAPGKNFQQAVQSALTKTHTRGPH
PSSVYGKPVIAAAQNQQQHPENIYSNSQGKPGSPEPETEPVSSVQENHENERIPRPLSPTKLLPFLSNPYRNQSDADLEA
LRKKLSNAPRPLKKRSSITBPEGPNGPNIQKLLYQRTTIAAMETISVPSYPSKSASVTASSESPVEIQNPYLHVEPEKEV
VSLVPESLSPEDVGNASTENSDMPAPSPGLDYEPEGVPDNSPNLQNNPEEPNPEAPHVLDVYLEBYPPYPPPPPSGEPE
GPGEDSVSMRPPBITGQVSLPPGKRTNLRKTGSERIAHGMRVKFNPLALLLDSSLEGEFDLVQRIIYEVDDPSLPNDEGI
TALHNAVCAGHTEIVKFLVQFGVNVNAADSDGWTPLHCAASCNNVQVCKFLVESGAAVFAMTYSDMQTAADKCEEMBEGY
KPRQRSLA

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Figure 18a

ATGACGGAATATAAGCTGGTGGTGGTGGGCGCCGGCGGTGTGGGCAAGA
GTGCGCTGACCATCCAGCTGATCCAGAACCATTTTGTGGACGAATACGAC
CCCACTATAGAGGATTCCTACCGGAAGCAGGTGGTCATTGATGGGGAGAC
GTGCCTGTTGGACATCCTGGATACCGCCGGCCAGGAGGAGTACAGCGCCA
TGCGGGACCAGTACATGCGCACCGGGGAGGGCTTCCTGTGTGTTTTGCC
ATCAACAACACCAAGTCTTTTGAGGACATCCACCAGTACAGGGAGCAGAT
CAAACGGGTGAAGGACTCGGATGACGTGCCCATGGTGCTGGTGGGGAAC
AAGTGTGACCTGGCTGCACGCACTGTGGAATCTCGGCAGGCTCAGGACCT
CGCCCGAAGCTACGGCATCCCCTACATCGAGACCTCGGCCAAGACCCGGC
AGGGAGTGGAGGATGCCTTCTACACGTTGGTGCGTGAGATCCGGCAGCAC
AAGCTGCGGAAGCTGAACCCTCCTGATGAGAGTGGCCCCGGCTGCATGAG
CTGCAAGTGTGTGCTCTCCTGA

Figure 18b

MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCL LDILDTAGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHQYREQIKRVK DSDDVPMVLVGNKCDLAARTVESRQAQDLARSYGIPYIETSAKTRQGVEDAF YTLVREIRQHKLRKLNPPDESGPGCMSCKCVLS

Figure 18c

ATGACGAATATAAGCTGGTGGTGGTGGGCGCCGTCGGTGTGGGCAAGA
GTGCGCTGACCATCCAGCTGATCCAGAACCATTTTGTGGACGAATACGAC
CCCACTATAGAGGATTCCTACCGGAAGCAGGTGGTCATTGATGGGGAGAC
GTGCCTGTTGGACATCCTGGATACCGCCGGCCAGGAGGAGTACAGCGCCA
TGCGGGACCAGTACATGCGCACCGGGGAGGGCTTCCTGTGTGTTTTGCC
ATCAACAACACCAAGTCTTTTGAGGACATCCACCAGTACAGGGAGCAGAT
CAAACGGGTGAAGGACTCGGATGACGTGCCCATGGTGCTGGTGGGGAAC
AAGTGTGACCTGGCTGCACGCACTGTGGAATCTCGGCAGGCTCAGGACCT
CGCCCGAAGCTACGGCATCCCCTACATCGAGACCTCGGCCAAGACCCGGC
AGGGAGTGGAGGATGCCTTCTACACGTTGGTGCGTGAGATCCGGCAGCAC
AAGCTGCGGAAGCTGAACCCTCCTGATGAGAGTGGCCCCGGCTGCATGAG
CTGCAAGTGTGTGCTCCTTGA

Figure 18d

MTEYKLVVVGAVGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCL LDILDTAGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHQYREQIKRVK DSDDVPMVLVGNKCDLAARTVESRQAQDLARSYGIPYIETSAKTRQGVEDAF YTLVREIRQHKLRKLNPPDESGPGCMSCKCVLS-

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Figure 18e

ATGACTGAATATAAACTTGTGGTAGTTGGAGCTGGTGGCGTAGGCAAGAG
TGCCTTGACGATACAGCTAATTCAGAATCATTTTGTGGACGAATATGATCC
AACAATAGAGGATTCCTACAGGAAGCAAGTAGTAATTGATGGAGAAACC
TGTCTCTTGGATATTCTCGACACAGCAGGTCAAGAGGAGTACAGTGCAAT
GAGGGACCAGTACATGAGGACTGGGGAGGGCTTTCTTTGTGTATTTGCCA
TAAATAATACTAAATCATTTGAAGATATTCACCATTATAGAGAACAAATT
AAAAGAGTTAAGGACTCTGAAGATGTACCTATGGTCCTAGTAGGAAATAA
ATGTGATTTGCCTTCTAGAACAGTAGACACAAAACAGGCTCAGGACTTAG
CAAGAAGTTATGGAATTCCTTTTATTGAAACATCAGCAAAAGACAAGACAG
GGTGTTGATGATGCCTTCTATACATTAGTTCGAGAAATTCGAAAAACATAA
AGAAAAGATGAGCAAAGATGGTAAAAAAGAAGAAAAAGAAGTCAAAGAC
AAAGTGTGTAATTATGTAA

Figure 18f

MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCL LDILDTAGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKRVK DSEDVPMVLVGNKCDLPSRTVDTKQAQDLARSYGIPFIETSAKTRQGVDDAF YTLVREIRKHKEKMSKDGKKKKKKSKTKCVIM-

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Figure 18g

ATGACTGAATATAAACTTGTGGTAGTTGGAGCTGTCGGCGTAGGCAAGAG
TGCCTTGACGATACAGCTAATTCAGAATCATTTTGTGGACGAATATGATCC
AACAATAGAGGATTCCTACAGGAAGCAAGTAGTAATTGATGGAGAAACC
TGTCTCTTGGATATTCTCGACACAGCAGGTCAAGAGGAGTACAGTGCAAT
GAGGGACCAGTACATGAGGACTGGGGAGGGCTTTCTTTGTGTATTTGCCA
TAAATAATACTAAATCATTTGAAGATATTCACCATTATAGAGAACAAATT
AAAAGAGTTAAGGACTCTGAAGATGTACCTATGGTCCTAGTAGGAAATAA
ATGTGATTTGCCTTCTAGAACAGTAGACACAAAACAGGCTCAGGACTTAG
CAAGAAGTTATGGAATTCCTTTTATTGAAACATCAGCAAAAGACAAGACAG
GGTGTTGATGATGCCTTCTATACATTAGTTCGAGAAATTCGAAAAACATAA
AGAAAAGATGAGCAAAAGATGGTAAAAAAGAAGAAAAAAGAAGTCAAAGAC
AAAGTGTGTAATTATGTAA

Figure 18h

MTEYKLVVVGAVGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCL LDILDTAGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKRVK DSEDVPMVLVGNKCDLPSRTVDTKQAQDLARSYGIPFIETSAKTRQGVDDAF YTLVREIRKHKEKMSKDGKKKKKKSKTKCVIM-

Figure 19a

atggcggcgg cggcgggc gggcgcgggc ccggagatgg tccgcgggca ggtgttcgac gtggggccgc gctacaccaa cctctcgtac atcggcgagg gcgcctacgg catggtgtgc tctgcttatg ataatgtcaa caaagttcga gtagctatca agaaaatcag cccctttgag caccagacct actgccagag aaccctgagg gagataaaaa tcttactgcg cttcagacat qaqaacatca ttggaatcaa tgacattatt cgagcaccaa ccatcgagca aatgaaagat qtatatatag tacaggacct catggaaaca gatctttaca agctcttgaa gacacaacac ctcagcaatg accatatctg ctattttctc taccagatcc tcagagggtt aaaatatatc cattrageta acgttetgea cegtgacete aageetteea acetgetget caacaceace tgtgatetea agatetgtga etttggeetg geeegtgttg eagateeaga eeatgateae acagggttcc tgacagaata tgtggccaca cgttggtaca gggctccaga aattatgttg aattccaagg gctacaccaa gtccattgat atttggtctg taggctgcat tctggcagaa atgettteta acaggeceat etttecaggg aageattate ttgaccaget gaaacacatt ttgggtattc ttggatcccc atcacaagaa gacctgaatt gtataataaa tttaaaagct aggaactatt tgctttctct tccacacaaa aataaggtgc catggaacag gctgttccca aatgetgact ccaaagetet ggacttattg gacaaaatgt tgacattcaa cccacacaag aggattgaag tagaacaggc tctggcccac ccatatctgg agcagtatta cgacccgagt gacgagccca tcgccgaagc accattcaag ttcgacatgg aattggatga cttgcctaag gaaaagctca aagaactaat ttttgaagag actgctagat tccagccagg atacagatct

Figure 19b

MAAAAAGAGPEMVRGQVFDVGPRYTNLSYIGEGAYGMVCSAYDNVNKVRV AIKKISPFEHQTYCQRTLREIKILLRFRHENIIGINDIIRAPTIEQMKDVYIVQDLME TDLYKLLKTQHLSNDHICYFLYQILRGLKYIHSANVLHRDLKPSNLLLNTTCDLKI CDFGLARVADPDHDHTGFLTEYVATRWYRAPEIMLNSKGYTKSIDIWSVGCILA EMLSNRPIFPGKHYLDQLKHILGILGSPSQEDLNCIINLKARNYLLSLPHKNKVPW NRLFPNADSKALDLLDKMLTFNPHKRIEVEQALAHPYLEQYYDPSDEPIAEAPFK FDMELDDLPKEKLKELIFEETARFQPGYRS

Figure 20a

1 tegggetgag gtteeeggge gggegggege ggagagaege gggaageagg ggetgggegg 61 gggtcgcggc gccgcagcta gcgcagccag cccgagggcc gccgccgccg ccgcccagcg 121 cgctccgggg ccgccggccg cagccagcac ccgccgcgcc gcagctccgg gaccggcccc 181 ggccgccgcc gccgcgatgg gcaacgccgc cgccgccaag aagggcagcg agcaggagag 241 cgtgaaagaa ttcttagcca aagccaaaga agattttctt aaaaaatggg aaagtcccgc 301 tcagaacaca gcccacttgg atcagtttga acgaatcaag accctcggca cgggctcctt 361 cgggcgggtg atgctggtga aacacaagga gaccgggaac cactatgcca tgaagatcct 421 cgacaaacag aaggtggtga aactgaaaca gatcgaacac accctgaatg aaaagcgcat 481 cctgcaagct gtcaactttc cgttcctcgt caaactcgag ttctccttca aggacaactc 541 aaacttatac atggtcatgg agtacgtgcc cggcggggag atgttctcac acctacggcg 601 gatcggaagg ttcagtgagc cccatgcccg tttctacgcg gcccagatcg tcctgacctt 661 tgagtatetg cactegetgg ateteateta cagggacetg aageeggaga atetgeteat 721 tgaccagcag ggctacattc aggtgacaga cttcggtttc gccaagcgcg tgaagggccg 781 cacttggacc ttgtgcggca cccctgagta cctggcccct gagattatcc tgagcaaagg 841 ctacaacaag gccgtggact ggtgggccct gggggttctt atctatgaaa tggccgctgg 901 ctaccogccc ttcttcgcag accagcccat ccagatctat gagaagatcg tctctgggaa 961 qqtqcqcttc ccttcccact tcagctctga cttgaaggac ctgctgcgga acctcctgca 1021 qqtaqatete accaaqeqet ttgggaacet caaqaatqqq qtcaacqata tcaagaacea 1081 caagtggttt gccacaactg actggattgc catctaccag aggaaggtgg aagctccctt 1141 cataccaaag tttaaaggcc ctggggatac gagtaacttt gacgactatg aggaagaaga 1201 aatcogggtc tocatcaatg agaagtgtgg caaggagttt totgagtttt aggggcatgc 1261 ctgtgccccc atgggttttc ttttttcttt tttcttttt ttggtcgggg gggtgggagg 1381 ccctccaggg ttagggggag caggaagccc agataatcag agggacagaa acaccagctg 1441 ctcccctca tccccttcac cctcctgccc cctctcccac ttttcccttc ctctttcccc 1501 acagececce ageceetcag eceteecage ceaettetge etgttttaaa egagtttete 1561 aactccagtc agaccaggtc ttgctggtgt atccagggac agggtatgga aagaggggct 1621 cacgettaac tecageceec acceacacec ceateceace caaccacagg ceceacttge 1681 taagggcaaa tgaacgaagc gccaaccttc ctttcggagt aatcctgcct gggaaggaga 1741 gatttttagt gacatgttca gtgggttgct tgctagaatt tttttaaaaa aacaacaatt 1801 taaaatetta tttaagttee accagtgeet ceeteeetee tteetetaet eecaceeete 1861 ccatgtcccc ccattcctca aatccatttt aaagagaagc agactgactt tggaaaggga 1921 ggcgctgggg tttgaacctc cccgctgcta atctcccctg ggcccctccc cggggaatcc 1981 tetetgecaa teetgegagg gtetaggeee etttaggaag eeteegetet ettttteeee 2041 aacagacctg tcttcaccct tgggctttga aagccagaca aagcagctgc ccctctccct 2101 gccaaagagg agtcatcccc caaaaagaca gagggggagc cccaagccca agtctttcct 2161 cccagcagcg tttcccccca actccttaat tttattctcc gctagatttt aacgtccagc 2221 cttccctcag ctgagtgggg agggcatccc tgcaaaaggg aacagaagag gccaagtccc 2281 cccaagccac ggcccggggt tcaaggctag agctgctggg gaggggctgc ctgttttact 2341 cacceaccag ettecgeete ecceatectg ggegeecete etceagetta getgteaget 2401 gtccatcacc tctcccccac tttctcattt gtgctttttt ctctcgtaat agaaaagtgg 2461 ggagccgctg gggagccacc ccattcatcc ccgtatttcc ccctctcata acttctcccc 2521 atcccaggag gagttctcag gcctggggtg gggccccggg tgggtgcggg ggcgattcaa 2581 cctgtgtgct gcgaaggacg agacttcctc ttgaacagtg tgctgttgta aacatatttg

Figure 20b

MGNAAAAKKGSEQESVKEFLAKAKEDFLKKWESPAQNTAHLDQFERIKTLGTGSFGRVMLVKHKETGNHY AMKILDKQKVVKLKQIEHTLNEKRILQAVNFPFLVKLEFSFKDNSNLYMVMEYVPGGEMFSHLRRIGRFS EPHARFYAAQIVLTFEYLHSLDLIYRDLKPENLLIDQQGYIQVTDFGFAKRVKGRTWTLCGTPEYLAPEI ILSKGYNKAVDWWALGVLIYEMAAGYPPFFADQPIQIYEKIVSGKVRFPSHFSSDLKDLLRNLLQVDLTK RFGNLKNGVNDIKNHKWFATTDWIAIYQRKVEAPFIPKFKGPGDTSNFDDYEEEEIRVSINEKCGKEFSE WO 2005/054862 PCT/GB2004/003899 32/39

Figure 21a

ATGTCCGACAGCGAGAAGCTCAACCTGGACTCGATCATCGGGCGCCTGCT GGAAGTGCAGGGCTCGCGGCCTGGCAAGAATGTACAGCTGACAGAGAAC CATTCTTCTGGAGCTGGAGGCACCCCTCAAGATCTGCGGTGACATACACG AGAGCAACTACCTCTTTCTGGGGGACTATGTGGACAGGGGCAAGCAGTCC TTGGAGACCATCTGCCTGCTGCCTGTATAAGATCAAGTACCCCGAGAA CTTCTTCCTGCTCGTGGGAACCACGAGTGTGCCAGCATCAACCGCATCTA TGGTTTCTACGATGAGTGCAAGAGACGCTACAACATCAAACTGTGGAAAA CCTTCACTGACTGCCTGCCCATCGCGGCCATAGTGGACGAA AAGATCTTCTGCTGCCACGGAGGCCTGTCCCCGGACCTGCAGTCTATGGA GCAGATTCGGCGGATCATGCGGCCCACAGATGTGCCTGACCAGGGCCTGC TGTGTGACCTGCTGTGGTCTGACCCTGACAAGGACGTGCAGGGCTGGGGC GAGAACGACCGTGGCGTCTCTTTTACCTTTGGAGCCGAGGTGGTGGCCAA GTTCCTCCACAAGCACGACTTGGACCTCATCTGCCGAGCACACCAGGTGG TAGAAGACGGCTACGAGTTCTTTGCCAAGCGGCAGCTGGTGACACTTTTC TCAGCTCCCAACTACTGTGGCGAGTTTGACAATGCTGGCGCCATGATGAG TGTGGACGAGACCCTCATGTGCTCTTTCCAGATCCTCAAGCCCGCCGACA AGAACAAGGGGAAGTACGGGCAGTTCAGTGGCCTGAACCCTGGAGGCCG ACCCATCACCCCACCCCGCAATTCCGCCAAAGCCAAGAAATAG

Figure 21b

MSDSEKLNLDSIIGRLLEVQGSRPGKNVQLTENEIRGLCLKSREIFLSQPILLEL EAPLKICGDIHGQYYDLLRLFEYGGFPPESNYLFLGDYVDRGKQSLETICLLL AYKIKYPENFFLLRGNHECASINRIYGFYDECKRRYNIKLWKTFTDCFNCLPIA AIVDEKIFCCHGGLSPDLQSMEQIRRIMRPTDVPDQGLLCDLLWSDPDKDVQ GWGENDRGVSFTFGAEVVAKFLHKHDLDLICRAHQVVEDGYEFFAKRQLVT LFSAPNYCGEFDNAGAMMSVDETLMCSFQILKPADKNKGKYGQFSGLNPGG RPITPPRNSAKAKK

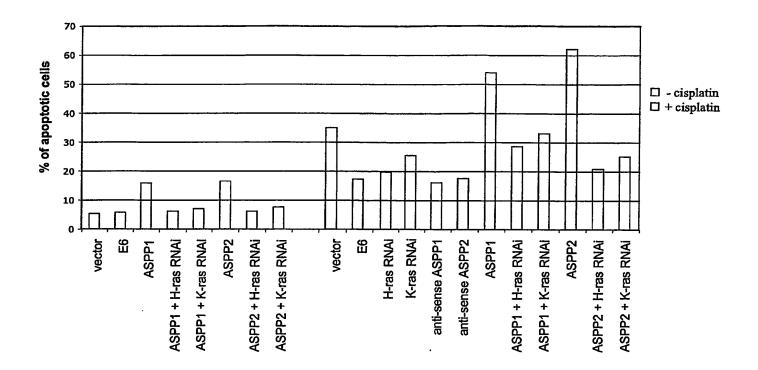
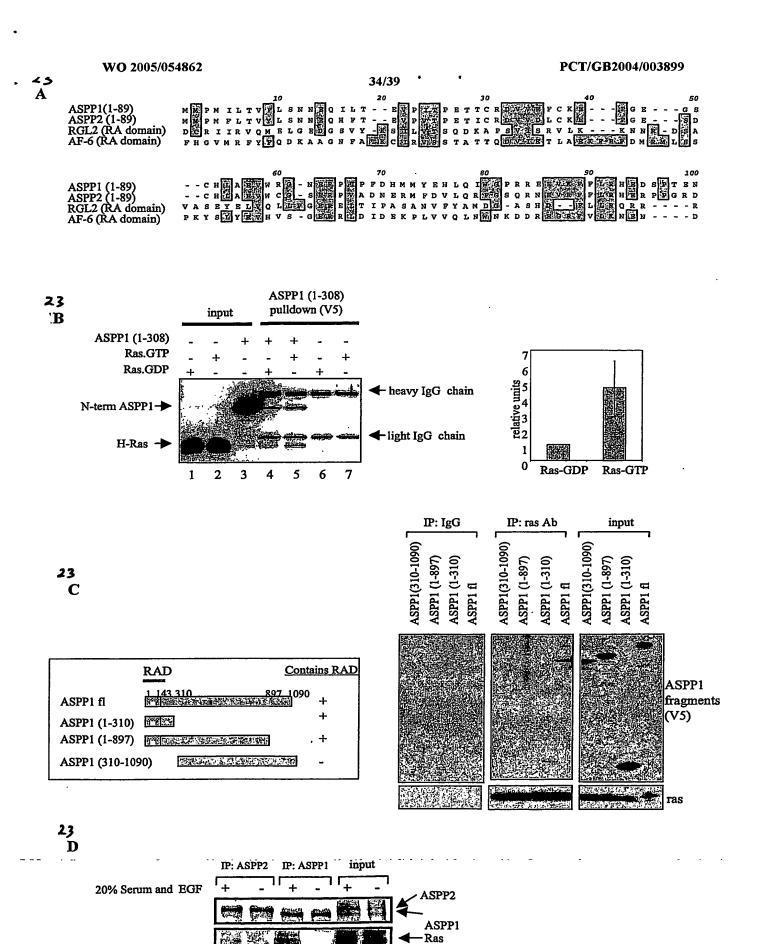


Figure 22



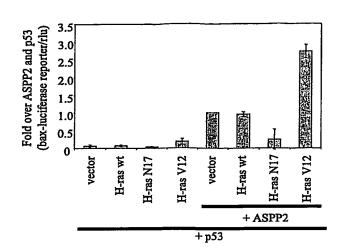
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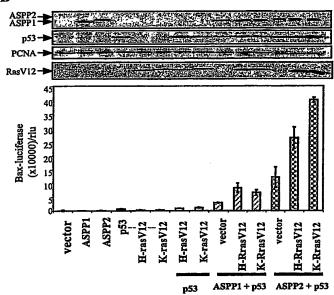
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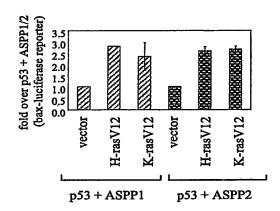
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24 A



24 B

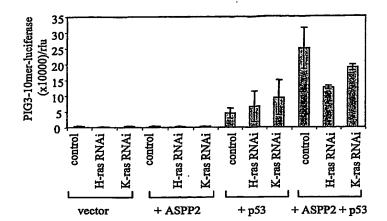




U2OS

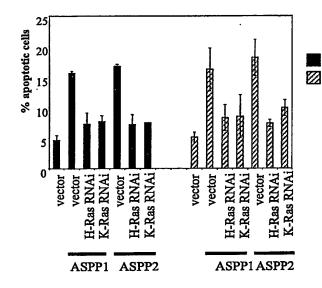
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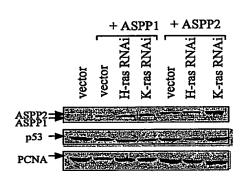
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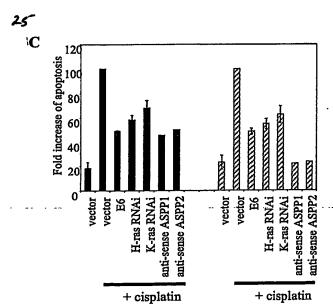


25

В







MAPK-P-

phospho-ASPP2→
Total ASPP2→

ASPP2 IP

0.5

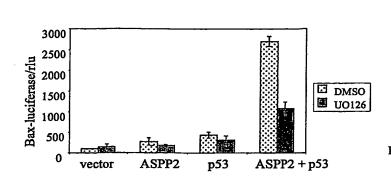
time after 20% FCS and

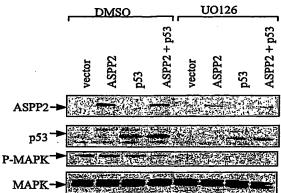
EGF stimulation / hour

Phospho-ASPP2

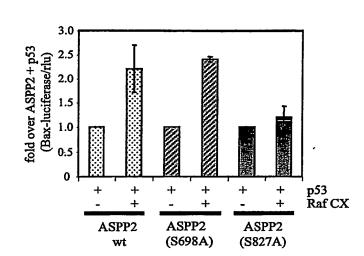
Unphospho-ASPP2-

27 · <u>A</u>

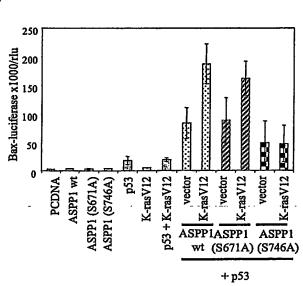


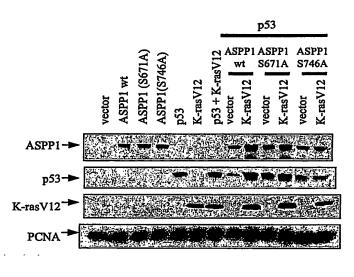


27 <u>B</u>



27 <u>C</u>





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